

WHAT IS CLAIMED IS:

1. A portable DNA sequence comprising a series of nucleotides capable of directing intracellular production of metalloproteinase inhibitors.

2. The portable DNA sequence of claim 1 wherein said sequence is capable of directing intracellular production of collagenase inhibitors.

3. The portable DNA sequence of claim 1 wherein said nucleotide sequence is:

10	20	30	40	50	60
GTTGTTGCTG TGGCTGATAG CCCCAGCAGG GCCTGCACCT GTGTCCCACC CCACCCACAC					
70	80	90	100	110	120
ACGGCCTTCT GCAATTCCGA CCTCGTCATC AGGGCCAAGT TCGTGGGGAC ACCAGAAAGT					
130	140	150	160	170	180
AACCAGACCA CCTTATACCA GCGTTATGAG ATCAAGATGA CCAAGATGTA TAAAGGGTT					
190	200	210	220	230	240
CAAGCCTTAG GGGATGCCGC TGACATCCGG TTCGTCTACA CCCCCGCCAT GGAGAGTGT					
250	260	270	280	290	300
TGCGGATACT TCCACAGGTC CCACAACCGC AGCGAGGAGT TTCTCATTCGC TGGAAAACT					
310	320	330	340	350	36
CAGGATGGAC TCTTGCACAT CACTACCTGC AGTTTCGTGG CTCCCTGGAA CAGCCTGAG					
370	380	390	400	410	42
TTAGCTCAGC GCCGGGGCTT CACCAAGACC TACACTGTTG GCTGTGAGGA ATGCACAGT					
430	440	450	460	470	48
TTTCCCTGTT TATCCATCCC CTGCAAACGT CAGAGTGGCA CTCATTGCTT GTGGACGGA					
490	500	510	520	530	54
CAGCTCCTCC AAGGCTCTGA AAAGGGCTTC CAGTCCCGTC ACCTTGCCTG CCTGCCTCG					
550	560	570	580	590	60
GAGCCAGGGC TGTGCACCTG GCAGTCCCTG CGGTCCCAGA TAGCCTGAAT CCTGCCCGG					

610            620            630            640            650            660  
 GTGGAAGCTG AAGCCTGCAC AGTGTCCACC CTGTTCCCAC TCCCCATCTTT CTTCCGGACA  
 670            680            690            700  
 ATGAAATAAAA GAGTTACCAAC CCAGAAAAAA AAAAAAGGAA TTC

4. The portable DNA sequence of claim 2 wherein said sequence is capable of directing intracellular production of a collagenase inhibitor biologically equivalent to that isolable from human skin fibroblasts.

5. A recombinant-DNA cloning vector comprising a nucleotide sequence capable of directing intracellular production of metalloproteinase inhibitors.

6. The vector of claim 5 wherein said vector comprises a nucleotide sequence containing at least the following nucleotides:

10	20	30	40	50	60
GTTGTTGCTG TGGCTGATAG CCCCAGCAGG GCCTGCACCT GTGTCCCACC CCACCCACAG					
70	80	90	100	110	120
ACGGCCTTCT GCAATTCCGA CCTCGTCATC AGGGCCAAGT TCGTGGGGAC ACCAGAACGTC					
130	140	150	160	170	180
AACCAGACCA CCTTATACCA GCGTTATGAG ATCAAGATGA CCAAGATGTA TAAAGGGTTC					
190	200	210	220	230	240
CAAGCCTTAG GGGATGCCGC TGACATCCGG TTCGTCTACA CCCCCGCCAT GGAGAGTGTC					
250	260	270	280	290	300
TGC GGATACT TCCACAGGTC CCACAACCGC AGCGAGGAGT TTCTCATTGC TGGAAAATG					
310	320	330	340	350	360
CAGGATGGAC TCTTGCACAT CACTACCTGC AGTTTGTGG CTCCCTGGAA CAGCCTGAGC					
370	380	390	400	410	420
TTAGCTCAGC GCCGGGGCTT CACCAAGACC TACACTGTG GCTGTGAGGA ATGCACAGTG					
430	440	450	460	470	480
TTTCCCTGTT TATCCATCCC CTGCAAACGT CAGAGTGGCA CTCATTGCTT GTGGACGGAC					

490 500 510 520 530 540  
CAGCTCCCTCC AAGGCTCTGA AAAGGGCTTC CAGTCCCCTC ACCTTGCCCTG CCTGCCCTCGA  
550 560 570 580 590 600  
GAGCCAGGGC TGTGCACCTG GCAGTCCCTG CGGTCCCAGA TAGCCTGAAT CCTGCCCGGG  
610 620 630 640 650 660  
GTGGAAGCTG AAGCCTGCAC AGTGTCCACC CTGTTCCCAC TCCCCATCTTT CTTCCGGAC  
670 680 690 700  
ATGAAATAAA GAGTTACCAAC CCAGCAAAAA AAAAAAGGAA TTC

7. The vector pUC9-F5/237P10.

8. A recombinant-DNA method for microbial production of a metalloproteinase inhibitor comprising:

- (a) preparation of a portable DNA sequence capable of directing a host microorganism to produce a protein having metalloproteinase inhibitor activity;
- (b) cloning the portable DNA sequence into a vector capable of being transferred into and replicating in a host microorganism, such vector containing operational elements for the portable DNA sequence;
- (c) transferring the vector containing the portable DNA sequence and operational elements into a host microorganism capable of expressing the metalloproteinase inhibitor protein;
- (d) culturing the host microorganism under conditions appropriate for amplification of the vector and expression of the inhibitor; and
- (e) in either order:
  - (i) harvesting the inhibitor; and
  - (ii) causing the inhibitor to assume an active, tertiary structure whereby it possesses metalloproteinase inhibitor activity.

9. The method of claim 8 wherein said metalloproteinase inhibitor is collagenase inhibitor.

10. The method of claim 8 wherein said portable DNA sequence is:

10            20            30            40            50            60  
GTTGTTGCTG TGGCTGATAG CCCCAGCAGG GCCTGCACCT GTGTCCCACC CCACCCACAG

70            80            90            100          110          120  
ACGGCCTTCT GCAATTCCGA CCTCGTCATC AGGGCCAAGT TCGTGGGGAC ACCAGAAGTC

130          140          150          160          170          180  
AACCAGACCA CCTTATACCA GCGTTATGAG ATCAAGATGA CCAAGATGTA TAAAGGGTTC

190          200          210          220          230          240  
CAAGCCTTAG GGGATGCCGC TGACATCCGG TTCGTCTACA CCCCCGCCAT GGAGAGTGTG

250          260          270          280          290          300  
TGC GGATACT TCCACAGGTC CCACAACCGC AGCGAGGAGT TTCTCATTGC TGGAAAACTG

310          320          330          340          350          360  
CAGGATGGAC TCTTGACAT CACTACCTGC AGTTTCGTGG CTCCCTGGAA CAGCCTGAGC

370          380          390          400          410          420  
TTAGCTCAGC GCCGGGGCTT CACCAAGACC TACACTGTTG GCTGTGAGGA ATGCACAGTG

430          440          450          460          470          480  
TTTCCCTGTT TATCCATCCC CTGCAAAC TG CAGAGTGGCA CTCATTGCTT GTGGACGGAC

490          500          510          520          530          540  
CAGCTCCTCC AAGGCTCTGA AAAGGGCTTC CAGTCCCCTC ACCTTGCCTG CCTGCCTCGG

550          560          570          580          590          600  
GAGCCAGGGC TGTGCACCTG GCAGTCCCTG CGGTCCCAGA TAGCCTGAAT CCTGCCCGGA

610          620          630          640          650          660  
GTGGAAGCTG AAGCCTGCAC AGTGTCCACC CTGTTCCCAC TCCCATCTTT CTTCCGGACA

670          680          690          700  
ATGAAATAAA GAGTTACAC CCAGCAAAAA AAAAAAGGAA TTC

11. The method of claim 8 wherein said vector containing said portable DNA sequence is pUC9-F5/237P10.

12. The method of claim 8 wherein said host microorganism is a bacterium.

13. The method of claim 12 wherein said bacterium is a member of the genus Bacillus.

14. The method of claim 13 wherein said bacterium is Bacillus subtilis.

15. The method of claim 12 wherein said bacterium is Escherichia coli.

16. The method of claim 12 wherein said bacterium is a member of the genus Pseudomonas.

17. The method of claim 16 wherein said bacterium is Pseudomonas aeruginosa.

18. The method of claim 8 wherein said host microorganism is a yeast.

19. The method of claim 8 wherein said yeast is Saccharomyces cerevisiae.

20. The method of claim 8 wherein said inhibitor is harvested prior to being caused to assume said active, tertiary structure.

21. The method of claim 8 wherein said inhibitor is caused to assume said active, tertiary structure prior to being harvested.

22. A metalloproteinase inhibitor which is biologically equivalent to the collagenase inhibitor isolable from human skin fibroblasts produced by the method of claim 8.

23. The microorganism C600/pUC9-F5/237P10 having ATCC Accession No. 53003.

24. The portable DNA sequence of claim 1 wherein said nucleotide sequence is:

10            20            30            40            50            6  
GGCCATGCC GCAGATCCAG CGCCCAGAGA GACACCAGAG AACCCACCAT GGCCCCCTT

70            80            90            100          110          12  
GACCCCTGGC TTCTGCATCC TGTTGTTGCT GTGGCTGATA GCCCCAGCAG GGCCTGCAC

130          140          150          160          170          18  
TGTGTCCCAC CCCACCCACA GACGGCCTTC TGCAATTCCG ACCTCGTCAT CAGGGCCAA

190          200          210          220          230          24  
TTCGTGGGA CACCAGAAAGT CAACCAGACC ACCTTATACC AGCGTTATGA GATCAAGATC

250          260          270          280          290          300  
ACCAAGATGT ATAAAGGGTT CCAAGCCTTA GGGGATGCCG CTGACATCCG GTTCGTCTAC

310          320          330          340          350          360  
ACCCCCGCCA TGGAGAGTGT CTGCGGATAC TTCCACAGGT CCCACAACCG CAGCGAGGAC

370          380          390          400          410          420  
TTTCTCATTG CTGGAAAACT GCAGGATGGA CTCTTGACACA TCACTACCTG CAGTTTCGTC

430          440          450          460          470          480  
GCTCCCTGGA ACAGCCTGAG CTTAGCTCAG CGCCGGGGCT TCACCAAGAC CTACACTGTT

490          500          510          520          530          540  
GGCTGTGAGG AATGCACAGT GTTCCCTGT TTATCCATCC CCTGCAAACCT GCAGAGTGGC

550          560          570          580          590          600  
ACTCATTGCT TGTGGACGGA CCAGCTCCTC CAAGGCTCTG AAAAGGGCTT CCAGTCCCGT

610          620          630          640          650          660  
CACCTTGCCT GCCTGCCTCG GGAGCCAGGG CTGTGCACCT GGCAGTCCCT GCGGTCCCAG

670          680          690          700          710          720  
ATAGCCTGAA TCCTGCCCGG AGTGGAAAGCT GAAGCCTGCA CAGTGTCCAC CCTGTTCCCA

730          740          750          760          770          780  
CTCCCACCTT TCTTCCGGAC AATGAAATAA AGAGTTACCA CCCAGCAAAA AAAAAAAGGA